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**REPORT FOR INHERENT BIODEGRADATION OF FRD903**

**[Modified MITI (II) Test]**

**Study No.: S2009NC031(a)-02**

**Report No.: R2009NC031(a)-02**

**Study Director: Shi Lili, professor**

**Date of Report Completion**

**Mar. 26, 2010**



**Key Lab. of Pesticide Environmental Assessment and Pollution Control, MEP**

**Address: 8 Jiang-wang-miao Street, Nanjing, 210042, China**

**Tel: (+86)25 85287074 Fax: (+86) 25 85474630**

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## SPONSOR AND TEST FACILITY

### Sponsor: E.I. du Pont de Nemours and Company

Address: CRP 702/1220, 4417 Lancaster Pike, Wilmington, DE 19805, USA

Tel: 001 302 999 3266

Sponsor's representative: Michael D. Bolten

E-Mail: michael.d.bolten@usa.dupont.com

### Test Substance: FRD903

### Test Facility: Key Lab of Pesticide Environmental Assessment & Pollution Control, MEP (PEAPC)

Address: 8 Jiangwangmiao Street, Nanjing 210042, China

Tel.: 86-25-85287074

Fax: 86-25-85474630

**Legal Representative Person:** Gao Zhenning, general-director, professor

**Laboratory Management:** Shan Zhengjun, professor

**Study Director:** Shi Lili, professor

**Study Personnel:** Liu Jining, Wang Na, Wu Guanqun

**Quality Assurance Personnel:** Ge Feng, assistant professor

## STATEMENT OF GLP COMPLIANCE

Study No.: S2009NC031(a)-02

Report No.: R2009NC031(a)-02

According to "OECD Guidelines for testing of chemicals" and "The guidelines for the testing of chemical (HJ/T 153-2004)" and "The guidelines of chemical testing good laboratory practices ((HJ/T 155-2004) " issued by State Environmental Protection Administration (SEPA) of the People's Republic of China, this experiment was conducted under CMA (China Metrology Accreditation) and CNAS (China National Accreditation Service for Conformity Assessment) experimental conditions at our laboratory. The experimental protocol was strictly carried out in the process of the experiment, and the present report has reflected the experimental results truly and correctly.

[Signature]  
(Study Director)

March 26, 2010  
Date:

[Signature]  
(Lab Management)

March 26, 2010  
Date:

## QUALITY ASSURANCE STATEMENT

Study No.: S2009NC031(a)-02

Report No.: R2009NC031(a)-02

This experiment was carried out strictly in accordance with the experimental protocol. It is hereby certified that what the present report describes has accurately reflected the raw data of the experiment.

The dates of Quality Assurance inspection are given below.

During the on-site process inspections procedures applicable to this type of study were inspected.

The reporting date is the date of reporting to the Study Director. The QAU report was then forwarded to the Test Facility Management.

Type of inspections	Phase/Process	Start inspection date	End inspection date	Reporting date
Study	Protocol Report	Dec. 8, 2009	Dec. 8, 2009	Dec. 8, 2009
		Jan. 28, 2010	Jan. 28, 2010	Jan. 28, 2010
Process	Test condition check & BOD measurements	Dec. 24, 2009	Dec. 24, 2009	Dec. 24, 2009

Gre Feng  
(Person responsible for QAU)

March 26, 2010  
Date:

## STUDY DETAILS PAGE

Study number:	S2009NC031(a)-02
Report number:	R2009NC031(a)-02
Study title:	Inherent Biodegradation: Modified MITI(II)
Test substance:	FRD 903
Identity:	FRD 903
CAS No.:	13252-13-6
Chemical name:	2, 3, 3, 3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid
Chemical formula:	$C_6HF_{11}O_3$
Molecular Weight:	330.05
Lot number:	--
Expiry date:	July, 2010
Appearance:	Liquid
Storage conditions:	Keep container tightly closed and store in a cool, dark, well ventilated location
Purity/Assay:	96%
Supplier:	E.I. du Pont de Nemours and Company
Head of Department:	Shan Zhengjun
Study Director:	Shi Lili
Person attending to routine duties and technical queries in the temporary absence of the Study Director:	Liu Jining.
Study Director contact details:	Shi Lili Telephone: +86 25 85287074 Facsimile: +86 25 85474630 Email: sll@nies.org, sllnes@hotmail.com
Location of study:	Key Lab of Pesticide Environmental Assessment and Pollution Control, MEP 8 Jiang-wang-miao Street Nanjing 210042 Jiangsu China
Study dates:	
Start:	Dec. 14, 2009
Completion:	Jan. 20, 2009
Draft report:	Feb. 03, 2009

## SUMMARY

The inherent biodegradation test for the test substance of FRD903 was conducted according to the following guidelines:

- 1) SEPA HJ/T 153-2004, "The guidelines for the testing of chemicals".
- 2) SEPA. The guidelines for the testing of chemicals. Beijing: China Environmental Sciences Publishing House. 2004.
- 3) GB/T 21818-2008. Chemical Inherent Biodegradation-Modified MITI Test (II).
- 4) OECD Procedure 302C, "Inherent Biodegradability: Modified MITI Test (II)".1981.

The inherent biodegradability of FRD903 was determined in a 28-day Biochemical Oxygen Demand (BOD) test and the analysis of residual chemical of FRD903 in BOD bottles in an aerobic, aqueous medium.

During the test, the temperature was kept at  $25\text{ }^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . The test was valid because the level of biodegradation of the reference substance aniline exceeded 40% after 7 days, and 65% after 14 days.

Based on the residue analysis, the biodegradation of FRD903 was 1.52% and there was hardly any change for the test chemical of FRD903 in the "abiotic" vessel during the testing period. The BOD results showed that biodegradation of FRD903 was both <1% after 14 days and 28 days.

Therefore, FRD903 has no inherently biodegradable under this test condition.



## 1 INTRODUCTION

The purpose of this test was to evaluate the inherent biodegradability of organic chemicals via a 28-day test. In the test, the test substance and/or micro-organisms not adapted to the test substance were added into the aerobic, aqueous medium in BOD bottles respectively. Then the Biochemical Oxygen Demand (BOD) and residual chemicals in BOD bottles was measured during the 28-day period. It was designed to meet the requirements of SEPA HJ/T 153-2004, "the guidelines for the testing of chemicals"; GB/T 21818-2008, "Chemical Inherent Biodegradation-Modified MITI Test (II)", and OECD Procedure 302C, "Inherent Biodegradability: Modified MITI Test (II)", adopted May 1981.

The method was not applicable to test substances that were inhibitory to aerobic sewage micro-organisms at the test concentration and that did not reach and react with the CO<sub>2</sub> adsorbent.

Test solutions were prepared in an inorganic salts medium, inoculated with a number of micro-organisms collected from not less than 10 places in Nanjing city. Those organisms collected were kept in BOD bottles in the dark at 25°C ± 1°C.

Four groups of "abiotic", "reference", "blank control" and "test" were set up simultaneously. The "abiotic" contained mineral salts medium and a measured amount of test substance in order to determine whether there was any change in the test chemical during the testing period, while the "reference" contained inoculated mineral salts medium and a measured amount of a reference substance for validating the test result. Besides the "control" only contained inoculated mineral salts medium, while the "test" contained inoculated mineral salts medium and a measured amount of the test substance.

The progress of degradation was followed by the determination BOD in the "test" and "control". Degradation was expressed as the ratio of the biochemical oxygen demand (BOD) and the theoretical oxygen demand (TOD) in order to evaluate the inherent biodegradability of chemical substance. Information on the relative proportions of the major components of the test substance together with their empirical formulae or TOD was therefore necessary prerequisites to this test.

The inherent degradation rate was also expressed as percentage of initial concentration of test substance, where the residue analysis of the test substance was performed at the beginning (0 d) and end (28 d) of the test.

Substances were considered to be "inherent biodegradable" if the inherent degradation rate was equal to or greater than 20% during the 28-day test period.

The test was invalid if the level of biodegradation of the reference substance did not exceed 40% after 7 days, and 65% after 14 days.

## 2 EQUIPMENT & MATERIALS

### 2.1 DATE OF TIME

Dec. 14, 2009 ~ Jan. 20, 2010

### 2.2 EQUIPMENTS & REAGENT

Incubator(Lovibond, GER), pH-electrodes, BOD bottles(500 mL), BOD meter (Lovibond, GER.Lovibond® BOD-OxiDirect sensor system for measurement of the Biochemical Oxygen Demand (BOD) measures BOD levels via the pressure drop in the closed system during a defined period of time. The inductive stirrer system not only mixes the sample but also ensures optimum gas exchange between sample and gas space in the sample flask), UPLC-MS-MS (Waters, USA).

Reagents for the medium were all analytical pure.

## 3 TEST DESIGN

### 3.1 PREPARATION OF THE INOCULUMS

Activated sludge, surface soil and surface water were sampled from ten sites distributed in four districts throughout Nanjing city, such as Chengdong, Chengbei, Baguazhou and Hexi. 1 L of the sludge, soil and water were collected and mixed thoroughly together. After removing floating matter, the mixture was allowed to stand and then the supernatant is filtrated through 0.45 µm Millipore filter. After that the filtrate was adjusted the supernatant to pH 7.0 with sodium hydroxide or phosphoric acid. Finally an appropriate volume of the filtered supernatant was transferred to a fill-and-draw activated sludge vessel and aerated for about 23.5 h.

Thirty minutes after stopping the aeration, about one third of the whole volume of supernatant was discarded. Then an equal volume of the solution (pH 7.0) containing 0.1% each of glucose, peptone and potassium orthophosphate, was added into the settled material and aerated again. This procedure was repeated once per day until the inoculums were used.

Before use the mixture was allowed to stand, and the supernatant was removed. A small quantity of sludge was taken to be centrifuged (10000 rpm×10 min) and then weighed. Then the sludge was dried in the oven and weighed again in order to calculate the content of dry sludge. At last a certain amount of centrifuged sludge was diluted with basal culture medium to get activated sludge suspension with a concentration of 1000 mg/L (dry basis).

### 3.2 PREPARATION OF SOLUTIONS OF THE TEST SUBSTANCE

A stock solution of the test substance (FRD903) at 100 mg/L: 105 mg of test substance was dissolved in 1 L volumetric flask with BSM.

A stock solution of the reference substance (aniline) at 1000 mg/L: 1001 mg of reference substance was dissolved in 1 L volumetric flask with BSM.

### 3.3 PREPARATION OF THE TEST MEDIUM

The Based Salt Medium (BSM) was prepared by adding 3 mL of each of the following stock solutions prepared in pre-aerated pure water to 1 litre of pure water.

	g/L
<b>Stock solution A</b>	
KH <sub>2</sub> PO <sub>4</sub> (potassium dihydrogen phosphate)	8.50
K <sub>2</sub> HPO <sub>4</sub> (dipotassium hydrogen phosphate)	21.8
Na <sub>2</sub> HPO <sub>4</sub> ·2H <sub>2</sub> O (disodium monohydrogen phosphate dihydrate)	22.2
NH <sub>4</sub> Cl (ammonium chloride)	1.7
The pH of this solution was 7.2	
<b>Stock solution B</b>	
CaCl <sub>2</sub> (calcium chloride)	27.5
<b>Stock solution C</b>	
MgSO <sub>4</sub> ·7H <sub>2</sub> O (magnesium sulphate heptahydrate)	22.5
<b>Stock solution D</b>	
FeCl <sub>3</sub> ·6H <sub>2</sub> O (iron (III) chloride hexahydrate)	0.25

### 3.4 TEST CONDITIONS

- (1) Concentration of test chemicals: 30 mg/L (W/V)
- (2) Concentration of reference chemicals: 100 mg/L (W/V)
- (3) Concentration of activated sludge: 100 mg/L (W/V)
- (4) Test temperature: 25°C ± 1°C
- (5) Period: 28 days
- (6) Stir vigorously with mechanical stirrer.

### 3.5 TEST PROCEDURE

15.0 mL of stock solution of the test substance were added to #1 vessel designated as "abiotic", then fixed 50 mL with BSM at 30 mg/L.

#2, #3 and #4 vessels of "test vessel" added 15.0 mL, 15.0 mL, 15.0 mL of stock solution of test substance respectively, then 5 mL of activated sludge suspension were added to every vessel and fixed 50 mL with BSM at 100 mg/L of activated sludge and 30 mg/L of test substance.

#5 vessel of "aniline" added 5 mL stock solution of reference substance and 5 mL of activated sludge suspension, then fixed 50 mL with BSM at 100 mg/L of activated sludge and 100 mg/L of reference substance.

#6 vessel of "blank control" added 5 mL of activated sludge suspension, and then fixed 50 mL with BSM at 100 mg/L of activated sludge.

After that, assembled the equipment, checked that it is air-tight, began the stirrers, and started the measurement of oxygen uptake under conditions of darkness.

The temperature, the operation of the stirrer and recorder was checked daily. Any changes in colour of the contents of the vessels were recorded. The BOD for the six bottles were determined and recorded at the days of 0, 5, 7, 11, 14, 18, 21, 25 and 28.

After the 0 and 28 days of testing, residual amounts of chemicals in the testing bottles were analysed.

### 3.6 CHEMICAL ANALYSIS

#### (1) Preparation of standard storage solution

A standard stock solution of 1000 mg/L FRD903: weighing 0.0521 g test substance and fixed 50 mL with deionized water.

Draw 1.0 mL standard stock solution of 1000 mg/L to 100 mL volumetric flask and fixed 100 mL with deionized water at 10.0 mg/L of test substance.

Draw 1.0 mL test substance solution at 10 mg/L to 10 mL volumetric flask and fixed 10 mL with deionized water at 1.0 mg/L of test substance.

#### (2) Preparation of work solutions

Draw 0.01, 0.05, 0.10, 0.20, 0.50 mL standard solution of test substance at 1.00 mg/L to 10.0 mL volumetric flask respectively, then fixed 10 mL with diluted 10000 times BSM, then obtained the standard work solutions at 0.001, 0.005, 0.01, 0.02, 0.05 mg/L. Details of the work solutions are showed as follows:

the concentration of the work solution (mg/L)	the concentration of the test solution added (mg/L)	the volume of the test solution added (mL)	fixed volume (mL)
0.001	1.00	0.010	10.0
0.005	1.00	0.050	10.0
0.01	1.00	0.10	10.0
0.02	1.00	0.20	10.0
0.05	1.00	0.50	10.0

#### (3) UPLC-MS-MS determination conditions

Apparatus: ACQUITY R™ Ultra Performance LC, Quattro Premier XE MS-MS (Waters, USA)

Column: ACQUITY UPLC® BEH C18 1.7 µm, 2.1×50 mm (Waters, USA)

Mobile phase: Methanol: water=60:40

Flow rate: 0.3 mL/min

Column Temperature: 30 °C



Injection volume: 10  $\mu$ L

MS/MS detection parameters are as follows:

Data type: SIR data

Ionisation mode: Electrospray, Negative

Capillary: 3.0 kV, Extractor: 3.0 V, RF Lens: 0.2 V

Source Temperature: 110  $^{\circ}$ C, Desolvation Temperature: 380  $^{\circ}$ C

Cone Gas Flow: 50 L/h, Desolvation Gas Flow: 500 L/h

LM Resolution 1: 7.9, LM Resolution 2: 5.1

HM Resolution 1: 15.0, HM Resolution 2: 14.7

Ion Energy 1: 0.4, Ion Energy 2: 2.0

Collision Gas (Pressure):  $3.43 \times 10^{-3}$  mbar

Ion (m/z) Dwell (s) Cone Volt. (V)

284.76 0.100 -31

Under the above conditions, the retention time of FRD903 was about 0.64 min (see Fig. 3).

(4) Sampling and analysis of test solution

Samples were taken from "abiotic" bottle at 0 day and 28 day, then from the "test" bottles at 28 day. After filtration by 0.22  $\mu$ m Millipore filter and diluted 10000-fold with BSM, the concentration of test substance was determined with the analysis method mentioned above.

#### 4 TEST VALIDITY

Viability of the microorganisms should be checked by means of a reference control. Biodegradation of the reference substance reached >40% and 65% on 7 day and 14 day.

#### 5 DATA PROCESSING

##### 5.1 CALCULATION OF THEORETICAL OXYGEN DEMAND

The theoretical oxygen demand (TOD) of the test and reference substance:



of molecular weight (MW), could be calculated in the following way assuming that carbon was mineralised to  $\text{CO}_2$ , hydrogen to  $\text{H}_2\text{O}$ , phosphorus to  $\text{P}_2\text{O}_5$  and potassium to  $\text{K}_2\text{O}$ . Halogens were presumed to be eliminated as hydrogen halides and nitrogen as ammonia.

$$\text{TOD}_{\text{NH}_3} = 16 [2c + 1/2 (h - \text{cl} - 3n) + 3s + 5/2p + 1/2k - o] \text{ (mgO}_2\text{/mg)}$$

MW

Nitrogen in a test substance could be eliminated as ammonia, nitrite or nitrate. The higher oxidation states gave a consequent increase in the  $\text{TOD} + 3n/2$  (nitrite) and  $+ 5n/2$  (nitrate).

Now, the molecular of test substance is  $\text{C}_6\text{HF}_{11}\text{O}_3$ . The  $\text{TOD}_{\text{NH}_3}$  of the test substance can be calculated as follow:

$$\text{TOD}_{\text{NH}_3} = \frac{16[2c + 1/2(h - f) - o]}{\text{MW}} = \frac{16[2 \times 6 + 1/2(1 - 11) - 3]}{330.05} = 0.194 \text{ (mgO}_2\text{/mg)}$$

The  $\text{TOD}_{\text{NH}_3}$  of the test substance FRD903 is 0.194 mg  $\text{O}_2$ /mg.

##### 5.2 CALCULATION OF PERCENTAGE BIODEGRADABILITY

(1) Method for calculating the percentage biodegradation from the oxygen consumption:

$$\% \text{ degradation} = \frac{\text{BOD} - B}{\text{TOD}} \times 100(\%)$$

BOD: Biological oxygen demand (experimental, mg) of the test substance measured on the BOD curve.

B: Oxygen consumption (experimental, mg) of basal culture medium to which the inoculum was added measured on the BOD curve.

TOD: Theoretical oxygen demand (theoretical, mg) required when the test substance is completely oxidised.

(2) Method for calculating the percentage degradation from the result of chemical analysis:

$$\% \text{ degradation} = \frac{S_b - S_a}{S_b} \times 100(\%)$$

$S_a$ : Residual amount (experimental, mg) of the test substance after completion of the biodegradability test.

$S_b$ : Residual amount (experimental, mg) of the test substance in the "abiotic" test to which only the test substance has been added.

#### 6 RESULTS

##### 6.1 ANALYTICAL METHOD OF FRD903 IN TEST SOLUTION

(1) Work curve

A series of work solutions with concentration at 0.001, 0.005, 0.01, 0.02, 0.05 mg/L were measured under the UPLC-MS-MS conditions mentioned above. Based on the test result, a linear regression equation was obtained with the concentration and the area of the peak emerged at



about 0.64 min:  $A = 388136c + 74.6$ , with good linearity of  $r^2=0.999$ , where  $A$  represents peak area; and  $c$  is concentration (mg/L) (see Fig. 2). The results show that linearity for concentration range of 0.001~0.5 mg/L is good.

#### (2) Detection limit

The minimum detection amount of UPLC-MS-MS for FRD903 is  $1.0 \times 10^{-11}$ g, and the minimum detection concentration for test sample is 0.001 mg/L.

### 6.2 INHERENT BIODEGRADATION OF FRD903

Table 1, Table 2, Table 3, Fig.4, Fig.5, Fig.6 showed results of inherent biodegradation data.

During the test, the temperature kept at  $25^\circ\text{C} \pm 1^\circ\text{C}$ . The test was valid because the level of biodegradation of the reference substance aniline exceeded 40% after 7 days, and 65% after 14 days.

The results showed that biodegradation of FRD903 were both  $<1\%$  after 14 days and 28 days.

Residual amounts (experimental, mg) of the test substance in "test" vessels after completion of the biodegradation test and in "abiotic" vessel before and after completion of the biodegradation test were determined by UPLC-MS-MS. Based on the residue analysis, the biodegradation of FRD903 was 1.52% and there was hardly any change of the test chemical in the "abiotic" vessel during the testing period.

Therefore, the test substance has no inherently biodegradable under this test condition.

### 7 RECORDS&DOCUMENTATION

All test samples arising from the performance of this study will remain the property of the Sponsor. Records and documentation relating to this study (including electronic records) will be maintained in the archives of PEAPC for a period of five year from the date on which the Director signs the final report. This includes raw data, a copy of the final report. Test substance remained will be retained by PEAPC in its archive for a period of one year from the date on which the Study Director signs the final report. After such time, the Sponsor will be contacted and his advice sought on the return, disposal or further retention of the materials. If requested, PEAPC will continue to retain the materials subject to a reasonable fee being agreed with the Sponsor.

### 8 HEALTH&SAFETY

In order for PEAPC to comply with Law of the People's Republic of China on the Prevention and Treatment of Occupational Diseases 2001, and the current Control of Substances Hazardous to Health Regulations, it is a condition of undertaking the study that the Sponsor provide PEAPC with all information available to it regarding known or potential hazards associated with the

handling and use of any substance supplied by the Sponsor to PEAPC. The Sponsor also complied with all current legislation and regulations concerning shipment of substances by road, rail, sea or air.

Such information in the form of a completed PEAPC test substance data sheet must be received at PEAPC before the test substance can be handled in the laboratory.

### 9 REFERENCES

- 1) SEPA. The guidelines for the testing of chemicals. HJ/T 153-2004. 2004.
- 2) SEPA. The guidelines for the testing of chemicals. Beijing: China Environmental Sciences Publishing House. 2004.
- 3) GB/T 21818-2008. Chemicals-Inherent biodegradability—Modified MITI test(II) . 2008.
- 4) OECD. 302C "Inherent Biodegradability: Modified MITI Test (II)". Paris: 1981.
- 5) SEPA. The guidelines for the hazard evaluation of new chemical substances. HJ/T 154-2004. 2004.
- 6) SEPA. The guidelines of chemicals testing good laboratory practices. HJ/T 155-2004. 2004.



## TABLES

**Table 1 Results of BOD**

Sample	BOD after n days (mg/L)								
	0 d	5 d	7 d	11 d	14 d	18 d	21 d	25 d	28 d
"abiotic"	1	5	8	9	9	10	16	22	22
"test"	9	14	17	26	31	37	40	46	52
	8	13	16	24	28	34	36	42	48
	8	14	17	26	30	37	39	46	52
"reference"	13	115	139	189	210	231	237	245	254
"blank control"	8	15	18	27	31	37	40	46	52

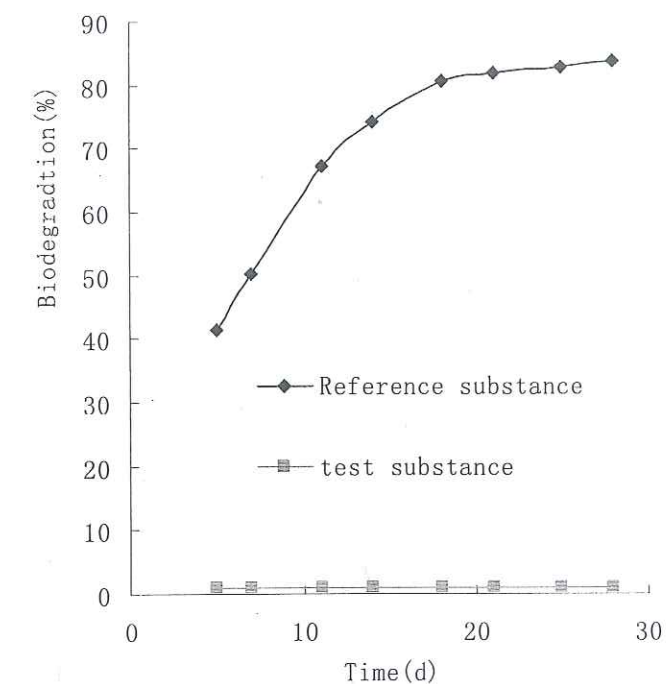
**Table 2 Biodegradation as BOD/TOD**

Sample		Biodegradation after n days (%)							
		5 d	7 d	11 d	14 d	18 d	21 d	25 d	28 d
Test substance (FRD903)	1	<1	<1	<1	<1	<1	<1	<1	<1
	2	<1	<1	<1	<1	<1	<1	<1	<1
	3	<1	<1	<1	<1	<1	<1	<1	<1
	mean	<1	<1	<1	<1	<1	<1	<1	<1
Reference (aniline)		41.5	50.2	67.2	74.3	80.5	81.7	82.6	83.8

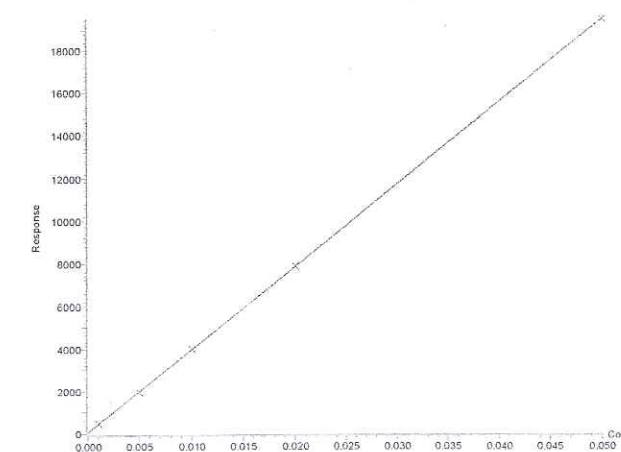
**Table 3 Analytical results and Degradation of FRD903**

sample	abiotic (0 day)	abiotic (28 days)	"test" (FRD903, 28days)		
Con. (mg/L)	31.1	30.8	29.5	31.3	30.7
Degradation (%)	-	-	4.22	0	0.325
Average (%)	-	-	1.52		

## FIGURES



**Figure 1 Biodegradation curve**



**Figure 2 Work curve**

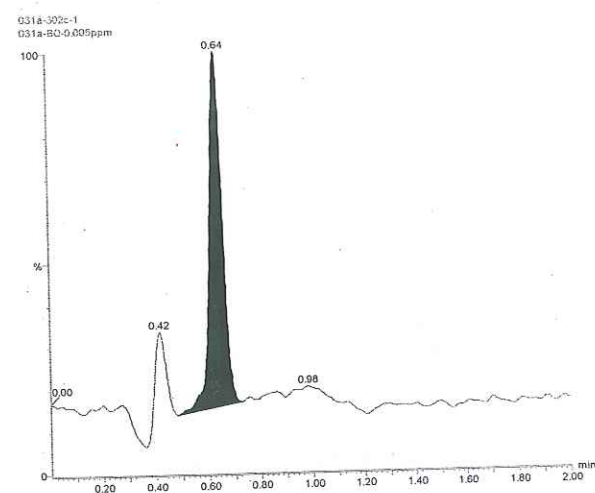
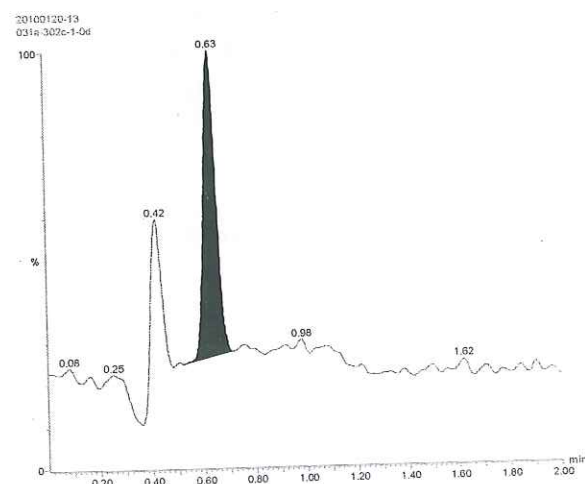
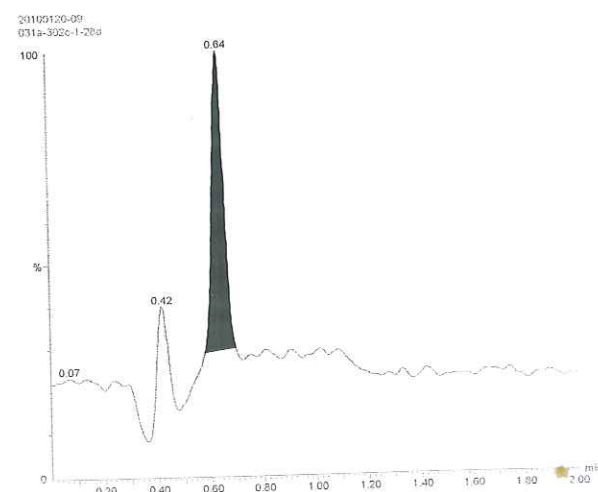


Figure 3 Chromatogram of FRD903 (0.005 mg/L)



0 d



28 d

Figure 4 Chromatogram of abiotic Sample at 0 d and 28 d

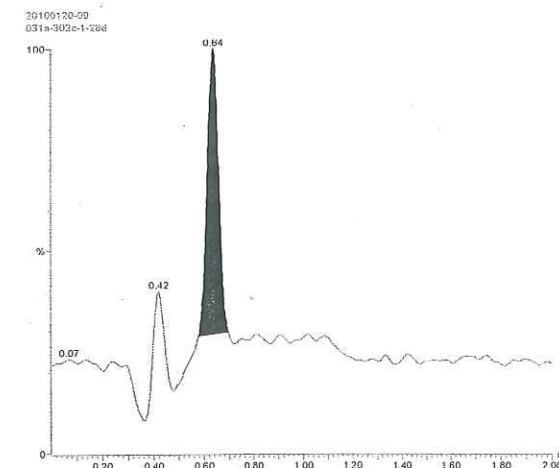


Figure 5 Chromatogram of Test Sample at 28 d

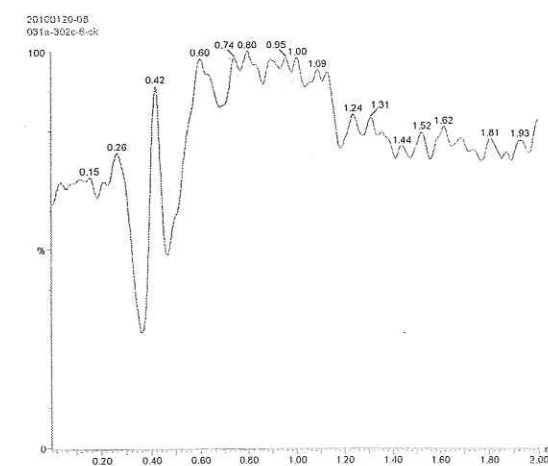


Figure 6 Chromatogram of Blank Control